REMARKS

Claims 1-17, 26-36, and 44-45 have been cancelled without prejudice, as these claims were non-elected in a restriction requirement. Claims 18-25, 37-43 and 46-51 are pending in the application, along with newly added Claims 52-107. Claims 18, 37 and 48 are independent claims. Newly added Claims 64 and 81 are also independent claims. Claims 19-25, along with newly added Claims 52-63, are dependent claims depending on independent Claim 18. Claims 38-43, 46-47, and 51, along with newly added Claims 96-107, are dependent claims depending on independent Claim 37. Claims 49-50 are dependent claims depending on independent Claim 48. Newly added dependent Claims 65-80 and 82-95 depend on newly added independent Claims 63 and 81, respectively. The "Summary of the Invention" section of the specification has been added to incorporate the specific language of newly added independent claims 63 and 81.

The Examiner has rejected Claims 48-50 under 35 U.S.C. Section 102(e) as being unpatentable over U.S. Patent No. 5,736,313 to Spargo, et al. Claims 18-25, 37-43, and 46-51 were rejected under 35 U.S.C. Section 103(a) as being unpatentable over U.S. Patent No. 5,736,313 to Spargo, et al in view of U.S. Patent No. 6,221,575 to Roser et al.

Applicants have amended independent Claims 18, 37, and 48 to patentably distinguish the same over the applied reference(s). As previously indicated, Applicants have added Claims 52-107 to patentably define further embodiments of the present invention.

Independent Claims 18, 37, and 48 as amended now claim that the platelets are loaded with an oligosaccharide (e.g., trehalose) by fluid phase endocytosis, which is patentably distinguishable over the teachings of over U.S. Patent No. 5,736,313 to Spargo, et al and U.S. Patent No. 6,221,575 to Roser et al, either taken singly or in combination. Spargo et al teaches no specific loading method. Roser et al teaches the loading of trehalose into platelets by one of the following methods (see column 5, lines 18-23 of Roser et al): "...electropermeabilisation, phase transition of the membrane, osmotic methods such as the use of organic osmolytes and pinocytosis, transient lysis methods such as acid shock and reversible cross-linking and the use of membrane permeable, esterase-labile trehalose derivatives." The Examiner is directed to page 9, lines 1-2 of the specification (".....trehalose uptake that seems to occur primarily through fluid phase endocytosis.....") for support in claiming fluid phase endocytosis.

Loading by electropermeabilisation as taught by Roser et al means or includes placing the cells in a strong electrical field, which opens transient pores in the plasma membrane, thereby allowing the trehalose to enter the platelets. This procedure also activates the platelets, thus limiting their usefulness. Loading by phase transition of the membrane as taught by Roser et al means or includes chilling the platelets through the main phospholipid phase transition (between 10-20^0 C) which makes the plasma membrane of some cells transiently leaky. In addition, such chilling of platelets induces cold-activation again limiting their clinical usefulness. This loading procedure

would not work for platelets. Loading by osmotic methods, such as the use of organic osmolytes, as taught by Roser et al, means or includes placing the cells in a hypertonic solution thus forcing the trehalose into the platelets down an osmotic gradient. Loading by transient lysis methods, such as acid shock and reversible cross-linking and the use of membrane permeable, esterase-labile trehalose derivatives, all as taught by Roser et al means or includes esterifying every hydroxyl group on the trehalose to make a hydrophobic group. All of those ester linkeages would then have to be cleaved in order for the trehalose to appropriately interact with the membranes for protection. The foregoing loading methods (i.e., electro-permeabilisation, loading by phase transition of the membrane, etc.) as taught by Roser et al is not the fluid phase endocytosis loading method as claimed by Applicants. The loading method taught by Roser et al which may be most related to the fluid phase endocytosis loading method as claimed by Applicants is loading by pinocytosis.

Loading by pinocytosis as taught by Roser et al includes non-specific formation of vesicles at the level of the plasma membrane. The vesicles formed by pinocytosis bud (or pinch) off into the cytoplasm, and subsequently leak nutrients (e.g., molecules) into the cytoplasm, without any fusing of vesicles with lysosomes. On the other hand, loading by the claimed fluid phase endocytosis for various embodiments of the present invention includes more specific formation of vesicles, requiring the involvement of a membrane-coat protein, such as clathrin. The vesicles formed by fluid phase endocytosis bud or pinch off into the cytoplasm, but subsequently fuse with lysosomes. In fluid

phase endocytosis there is no leaking of nutrients (e.g., molecules) into the cytoplasm, whereas, as indicated, in pinocytosis nutrients leak from the vesicles into the cytoplasm. Thus, the loading of an oligoshaccaride into platelets by fluid phase endocytosis for various embodiments of the present invention is distinct from the loading by pinocytosis as taught in U.S. Patent No. 6,221,575 to Roser et al.

As previously mentioned, Applicants have added independent Claims 64 and 81, and dependent Claims 52-63, 65-80, and 82-107, to claim additional embodiments of the present invention. Independent Claim 64 claims *inter alia* preventing a decrease in a loading efficiency gradient in loading of the oligosaccharide into the platelets. Independent Claim 81 claims *inter alia* preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets. These claimed features are not taught or suggested by Spargo et al and Roser et al, either taken singly or in combination.

Support in the specification for newly added claims may be found as follows: (i)page 18, lines 17-24 and Figure 3 for illustrating preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets; and an increase in loading efficiency for an oligosaccharide concentration up to about 50 mM (support for Claims 52, 57, 64, 65, 77 and 82); (ii)Figure 4 for illustrating preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets; and a loading efficiency ranging from about 45% to about 50% for the oligosaccharide solution having an

oligosaccharide concentration ranging from about 20 mM to about 30 mM (support for Claims 53, 68, 69, 78, 81, 85, 86, and 97); (iii) page 13, lines 8-10 and page 20, lines 14-15 for teachings of prehydrating lyophilized cooled platelets with moisture saturated air up to a water content ranging from about 35% to about 50% by weight and rehydrating prehydrated platelets (support for Claims 60-63, 73-76, 90-93, and 104-107); and (iv) page 9, lines 1-2 for loading by fluid phase endocytosis (support for Claims 66, 67, 83 and 84).

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned **"Version With Marking to Show Changes Made"**. Accompanying this Response is a Second Supplemental Disclosure Statement.

All Claims are now in condition for allowance and an early notice of same is respectfully solicited.

Respectfully Submitted

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CERTIFICATION OF MAILING

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IN THE SPECIFICATION

After the Paragraph on page 4 ending with "..the desired therapeutic application." at line 10, the following paragraphs have been added:

Embodiments of the present invention provide a process for preparing a dehydrated composition comprising disposing platelets in an oligosaccharide solution for loading an oligosaccharide from the oligosaccharide solution into the platelets, preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets, and lyophilizing the platelets. The preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets may comprise maintaining a concentration of the oligosaccharide in the oligosaccharide solution below about 50 mM. The preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets may also comprise maintaining a positive gradient of loading efficiency (%) to concentration (mM) of the oligosaccharide in the oligosaccharide solution.

Embodiments of the present invention also provide a process for preparing a dehydrated composition comprising disposing platelets in an oligosaccharide solution for loading an oligosaccharide from the oligosaccharide solution into the platelets, preventing a decrease in a loading gradient in the loading of the oligosaccharide into

the platelets, and lyophilizing the platelets. The preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets may comprise maintaining a concentration of the oligosaccharide in the oligosaccharide solution below about 50 mM. The preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets may also comprise maintaining a positive gradient of concentration of oligosaccharide loaded into the platelets to concentration of the oligosaccharide in the oligosaccharide solution.

IN THE CLAIMS

Claims 1-17, 26-36, and 44-45 have been cancelled without prejudice.

Claim 18 has been amended as follows:

18. (Twice Amended) A process of preparing a dehydrated composition comprising:

providing platelets selected from a mammalian species, the platelets being effectively loaded by fluid phase endocytosis with an oligosaccharide therein to preserve biological properties, wherein the loading includes incubating the platelets at a temperature from about 30°C to less than about 40°C with an oligosaccharide solution, the solution having up to about 50 mM oligosaccharide therein,

the incubating sufficient to load oligosaccharide inside the platelets in an amount from about 10 mM to about 50 mM;

cooling the loaded platelets to below their freezing point; and,

lyophilizing the cooled platelets.

Claim 37 has been amended as follows:

37. (Once Amended) A process for preparing a dehydrated composition comprising:

loading internally by fluid phase endocytosis platelets with from about 10 mM to about 50 mM oligosaccharide to produce internally loaded platelets; cooling the internally loaded platelets to below their freezing point; and lyophilizing the cooled internally loaded platelets.

Claim 48 has been amended as follows:

48. (Once Amended) A process for preparing a dehydrated composition comprising:

loading internally by fluid phase endocytosis platelets with a protectorate to produce internally loaded platelets; preventing the internally loaded platelets from activating; cooling the internally loaded platelets to

below their freezing point; and lyophilizing the cooled internally loaded platelets.

The following Claims have been added:

52. The process of Claim 18 wherein said loading with an oligosaccharide includes increasing a loading efficiency of the oligosaccharide into the platelets by maintaining a concentration of the oligosaccharide in the oligosaccharide solution at less than about 50 mM.

53. The process of Claim 18 wherein said loading with an oligosaccharide includes loading with a loading efficiency ranging from about 45% to about 50 % for the oligosaccharide solution having an oligosaccharide concentration ranging from about 20 mM to about 30 mM.

54. The process of Claim 18 wherein said oligosaccharide comprises trehalose.

55. The process of Claim 52 wherein said oligosaccharide comprises trehalose.

56. The process of Claim 53 wherein said oligosaccharide comprises trehalose.

57. The process of Claim 18 wherein said loading with an oligosaccharide includes decreasing a loading efficiency of the oligosaccharide into the platelets by providing a concentration of the oligosaccharide in the oligosaccharide solution at greater than about 50 mM.

58. The process of Claim 57 herein said oligosaccharide comprises trehalose.

59. The process of Claim 18 wherein said loading is without a fixative.

60. The process of Claim 18 additionally comprising prehydrating the lyophilized cooled platelets.

61. The process of Claim 60 wherein said prehydrating comprises exposing the lyophilized cooled platelets to moisture saturated air.

62. The process of Claim 18 additionally comprising prehydrating the lyophilized cooled platelets until the water content of the lyophilized cooled platelets ranges from about 35 % by weight to about 50 % by weight.

63. The process of Claim 60 additionally comprising rehydrating the prehydrated lyophilized cooled platelets.

64. A process for preparing a dehydrated composition comprising:

disposing platelets in an oligosaccharide solution for loading an oligosaccharide from the oligosaccharide solution into the platelets;

preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets; and

lyophilizing the platelets.

65. The process of Claim 64 wherein said preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets comprises maintaining a concentration of the oligosaccharide in the oligosaccharide solution below about 50 mM.

66. The process of Claim 64 wherein said loading comprises loading by fluid phase endocytosis.

67. The process of Claim 65 wherein said loading comprises loading by fluid phase endocytosis.

68. The process of Claim 64 wherein said loading with an oligosaccharide includes loading with a loading efficiency ranging from about 45% to about 50 % for the oligosaccharide solution having an oligosaccharide concentration ranging from about 20 mM to about 30 mM.

69. The process of Claim 66 wherein said loading with an oligosaccharide includes loading with a loading efficiency ranging from about 45% to about 50% for the oligosaccharide solution having an oligosaccharide concentration ranging from about 20 mM to about 30 mM.

70. The process of Claim 64 wherein said oligosaccharide comprises trehalose.

71. The process of Claim 67 wherein said oligosaccharide comprises trehalose.

72. The process of Claim 64 wherein said loading is without a fixative.

73. The process of Claim 64 additionally comprising prehydrating the lyophilized platelets.

74. The process of Claim 73 wherein said prehydrating comprises exposing the lyophilized platelets to moisture saturated air.

75. The process of Claim 64 additionally comprising prehydrating the lyophilized platelets until the water content of the lyophilized platelets ranges from about 35 % by weight to about 50 % by weight.

76. The process of Claim 73 additionally comprising rehydrating the prehydrated lyophilized platelets.

77. The process of Claim 64 wherein said preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets comprises maintaining a positive gradient of loading efficiency to concentration of the oligosaccharide in the oligosaccharide solution.

78. The process of Claim 64 wherein said preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the platelets comprises maintaining a positive gradient of loading efficiency (%) to concentration (mM) of the oligosaccharide in the oligosaccharide solution.

79. The process of Claim 77 wherein said oligosaccharide comprises trehalose.

80. The process of Claim 78 wherein said oligosaccharide comprises trehalose.

81. A process for preparing a dehydrated composition comprising:

disposing platelets in an oligosaccharide solution for loading an oligosaccharide from the oligosaccharide solution into the platelets;

preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets; and

lyophilizing the platelets.

82. The process of Claim 81 wherein said preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets comprises maintaining a concentration of the oligosaccharide in the oligosaccharide solution below about 50 mM.

83. The process of Claim 81 wherein said loading comprises loading by fluid phase endocytosis.

84. The process of Claim 82 wherein said loading comprises loading by fluid phase endocytosis.

85. The process of Claim 81 wherein said loading with an oligosaccharide includes loading with a loading efficiency ranging from about 45% to about 50 % for the oligosaccharide solution having an oligosaccharide concentration ranging from about 20 mM to about 30 mM.

86. The process of Claim 83 wherein said loading with an oligosaccharide includes loading with a loading efficiency ranging from about 45% to about 50% for the oligosaccharide solution having an oligosaccharide concentration ranging from about 20 mM to about 30 mM.

87. The process of Claim 81 wherein said oligosaccharide comprises trehalose.

88. The process of Claim 84 wherein said oligosaccharide comprises trehalose.

89. The process of Claim 81 wherein said loading is without a fixative.

90. The process of Claim 81 additionally comprising prehydrating the lyophilized platelets.

91. The process of Claim 90 wherein said prehydrating comprises exposing the lyophilized platelets to moisture saturated air.

92. The process of Claim 81 additionally comprising prehydrating the lyophilized platelets until the water content of the lyophilized platelets ranges from about 35 % by weight to about 50 % by weight.

93. The process of Claim 90 additionally comprising rehydrating the prehydrated lyophilized platelets.

94. The process of Claim 81 wherein said preventing a decrease in a loading gradient in the loading of the oligosaccharide into the platelets comprises maintaining a positive gradient of concentration of oligosaccharide loaded into the platelets to concentration of the oligosaccharide in the oligosaccharide solution.

95. The process of Claim 94 wherein said oligosaccharide comprises trehalose.

96. The process of Claim 37 wherein said loading with an oligosaccharide includes increasing a loading efficiency of the oligosaccharide into the platelets by maintaining a concentration of the oligosaccharide in the oligosaccharide solution at less than about 50 mM.

97. The process of Claim 37 wherein said loading with an oligosaccharide includes loading with a loading efficiency ranging from about 45% to about 50 % for the oligosaccharide solution having an oligosaccharide concentration ranging from about 20 mM to about 30 mM.

98. The process of Claim 37 wherein said oligosaccharide comprises trehalose.

99. The process of Claim 96 wherein said oligosaccharide comprises trehalose.

100. The process of Claim 97 wherein said oligosaccharide comprises trehalose.

101. The process of Claim 37 wherein said loading with an oligosaccharide includes decreasing a loading efficiency of the oligosaccharide into the platelets by providing a concentration of the oligosaccharide in the oligosaccharide solution at greater than about 50 mM.

102. The process of Claim 101 herein said oligosaccharide comprises trehalose.

103. The process of Claim 37 wherein said loading is without a fixative.

104. The process of Claim 37 additionally comprising prehydrating the lyophilized cooled platelets.

105. The process of Claim 104 wherein said prehydrating comprises exposing the lyophilized cooled platelets to moisture saturated air.

106. The process of Claim 37 additionally comprising prehydrating the lyophilized cooled platelets until the water content of the lyophilized cooled platelets ranges from about 35 % by weight to about 50 % by weight.

107. The process of Claim 104 additionally comprising rehydrating the prehydrated lyophilized cooled platelets.